# Separation and Characterization of Cells with Varying Magnetic Nanoparticle Concentration

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**Background:** The activation of magnetic nanoparticles (mNPs) by an alternating magnetic field (AMF) is currently being explored as technique for targeted therapeutic heating of tumors.[1] Engineered nanoparticles have attracted much attention during the last decade because of their size-related properties, large surface-to-volume ratios and high surface reactivity. Parameters such as the size, charge and surface chemistry are examined because they affect the interactions of the nanoparticles with living cells.[2] The mechanism for the uptake and distribution within cells is not fully understood. Optimization of targeted cellular uptake is critical if many nanoparticle-based applications are to become utilized in the clinical and research settings.

**Objective:** We propose to develop a method to separate cells based on intracellular mNP concentration which will enable further study and optimization of cellular uptake in tumor cells.

**Proof of Principle:** In order to demonstrate that cells containing mNPs could be moved with magnets we conducted the following test. The particles used for these studies are BNF-Dextran plain, 84-00-102, (micromod GmbH, Rostock, Germany). These particles have a hydrodynamic radius of 100 nm, and are suspended in water at 25 mg/ml. 0.120 mL of mNP were added to 3 mL of cell culture media to a flask of Chinese hamster ovary (CHO) cells. Cells were incubated for 4 days, after which the media was removed and cells were washed with PBS solution to remove extracellular mNP. Trypsin was added to the flask to cleave cells from the bottom of the flask. A cell pellet was created with centrifugation (Figure A). Cells were re-suspended in fresh medium in a six well plate, with rare earth magnets placed beneath them (Figure B). After 24 hrs, the media was removed and dark ring was observed where the magnets had been placed (Figure C). Methylene blue dye was added to stain the sells. Cells were seen to show strong attraction to the magnet (Figure D).

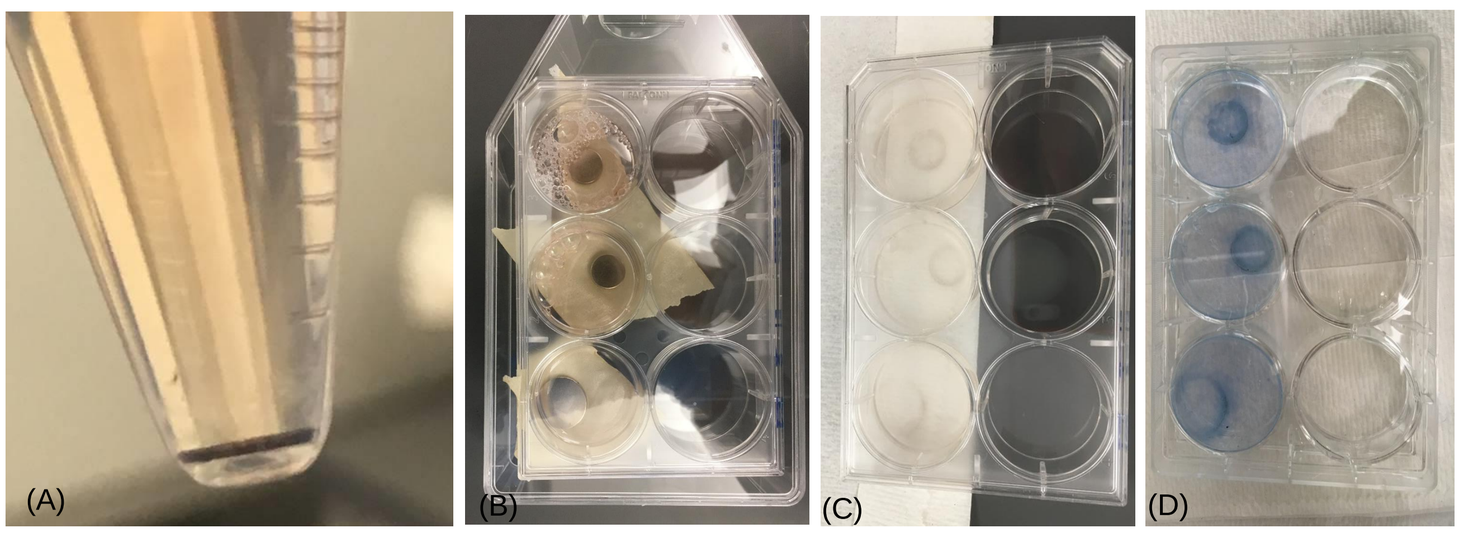


**Figure A: Cell pellet is black in color which indicates the presence of mNP in cells.**
**Figure B: Magnets placed under six well plate.**
**Figure C: Dark circles suggests the presence of mNP where the magnets had been placed.**
**Figure D: methylene blue stain (indicates cells) suggests higher cell concentration where the magnets had been placed.**

**Method of quantifying mNP:** Once we determined that cells containing mNP could be moved with magnets, we sought to quantify the amount of mNP in the cells with UV-Vis spectroscopy. Aliquots of cells were digested with HCL and a range of mNP concentrations. Using a microplate reader we demonstrated that the absorbance varied most significantly between our concentrations of interest with mNP concentration within 240-250nm.

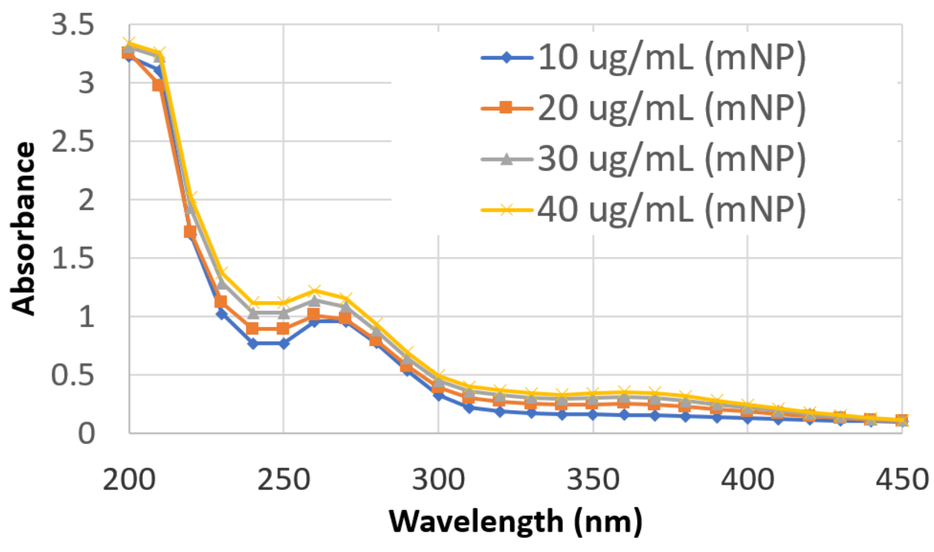


**Figure E: Variation of absorbance with wavelength and mNP concentration. Data suggests that mNP concentration can be determined by changes in absorbance, particularly in the 240-250 nm wavelength.**

**Next generation cell separator:** In this experiment we are planning to add BNF- Dextran coated IONP's in the CHO cells. The cells will incubated for 72 hrs after which the cells will be washed with PBS to remove the extracellular mNP's. Cells will be centrifuged to form a cell pellet and resuspended in a six well plate with rare earth magnets attached underneath the wells. After 24 hrs, a dark ring around the magnets should form, the media will be removed and the cells which form the ring will be scraped off the surface by a cell scraper and will be resuspended in a conical tube containing media. The cells will be further centrifuged to form a pellet. The media is aspirated and the pellet is kept to dry at room temp for overnight. After the cell pellet is dry, it will be treated with 1ml HCL and heated at 60C for 4 hrs. After the digestion is complete, the absorbance will be measured between 200-800nm and the absorbance will be normalized to find iron content in each cell sub-population.

**Significance:** Current methods of mNP quantification consider the average iron concentration in a bulk sample (millions of cells), or are qualitative/2D methods which do not provide absolute iron values. By developing a method which separates living cells, we will be able better examine the mechanisms responsible for intracellular uptake. In addition, we will be able to quantify the variation of mNP concentration cell to cell which histology suggests exists, ultimately improving particle design and their application in a broad variety of medical applications.

## References:

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